AMRL-TR-72-94

BERYLLIUM UPTAKE BY THE COMMON GUPPY USING RADIOISOTOPE ^{7}Be.

I. PRELIMINARY STUDY: TRACER MECHANICS AND PROCEDURES

FREDERICK C. DAMM, CAPTAIN, USAF
AIR FORCE INSTITUTE OF TECHNOLOGY

ARNOLD R. SLONIM, PhD
AEROSPACE MEDICAL RESEARCH LABORATORY

OCTOBER 1972

Approved for public release; distribution unlimited.

AEROSPACE MEDICAL RESEARCH LABORATORY
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO

NOTICES

When US Government drawings, specifications, or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Organizations and individuals receiving announcements or reports via the Aerospace Medical Research Laboratory automatic mailing lists should submit the addressograph plate stamp on the report envelope or refer to the code number when corresponding about change of address or cancellation.

Do not return this copy. Retain or destroy.

Please do not request copies of this report from Aerospace Medical Research Laboratory. Additional copies may be purchased from:

National Technical Information Service
5285 Port Royal Road
Springfield, Virginia 22151

The experiments reported herein were conducted according to the "Guide for Laboratory Animal Facilities and Care," prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences—National Research Council.

AIR FORCE/56780/30 March 1973 — 100

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)*
Aerospace Medical Research Laboratory
Aerospace Medical Div., Air Force Systems Command
Wright-Patterson Air Force Base, OH 45433

2a. REPORT SECURITY CLASSIFICATION
UNCLASSIFIED

2b. GROUP
N/A

3. REPORT TITLE

Beryllium Uptake by the Common Guppy Using Radioisotope ^{7}Be. I. Preliminary Study: Tracer Mechanics and Procedures.

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*
Final Report, 1970-1971

5. AUTHOR(S) *(First name, middle initial, last name)*

Frederick C. Damm, Captain, USAF
Arnold R. Slonim, PhD

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
October 1972	iii + 14	6

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. 6302	AMRL-TR-72-94
c. Task No. 630204	9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
d. Work Unit No. 630204013	

10. DISTRIBUTION STATEMENT

Approved for public release; distribution unlimited.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	Aerospace Medical Research Laboratory Aerospace Medical Div., Air Force Systems Command, Wright-Patterson AFB, OH 45433

13. ABSTRACT

The mechanics and several variables concerning the use of radioactive beryllium, Be-7, and the possible interaction between beryllium solutions and glass were studied prior to the application of the isotope (in follow-on phase) as a monitor of beryllium toxicity in guppies. Radioactivity was measured in a scintillation counter incorporating a sodium iodide well detector. Tracer activity or efficiency was evaluated under various conditions (e.g., isotope concentration, storage in glass over time, and counting geometry relative to sample size and position in detector). Tracer activity was proportional to tracer concentration and unaltered in a glass container for a period up to 8 days. Under the conditions of this study, beryllium was not lost from BeSO4 solutions in glass bioassay jars due to adsorption onto the glass or evaporation. Counting efficiency was relatively independent of sample size or position within well detector, so that water samples and exposed fish can be treated alike relative to the detector geometry. A procedure was outlined for estimating beryllium uptake in fish exposed to aqueous beryllium solutions to which tracer has been added.

DD FORM 1 NOV 65 1473

Security Classification

Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Beryllium						
Radioactive Beryllium (^{7}Be)						
$BeSO_4$ Solution						
Beryllium Toxicity						
Scintillation Counter						
Beryllium Uptake						
Fish (Guppies)						

Security Classification

FOREWORD

This study was accomplished in support of Project 6302, "Toxic Hazards of Propellants and Materials," Task 630204, "Environmental Pollution Aspects of Propellants and Materials." The research covered in this report was conducted during the first half of 1970, utilizing mainly the facilities at the Nuclear Engineering Center (NEC) of the Air Force Institute of Technology at Wright-Patterson Air Force Base. This phase of the project was completed by Captain Frederick C. Damm,* Engineering and Experimentation Division, NEC, AFIT, WPAFB, and Dr. A. R. Slonim, Toxic Hazards Division, Aerospace Medical Research Laboratory, WPAFB.

The authors are deeply grateful to Mr. Anthony N. Fasano, formerly Chief, Engineering and Experimentation Division, Nuclear Engineering Center, AFIT, for his role in the planning, arranging and safety aspects of this joint effort, his assistance in the acquisition of essential materials and facilities to do the work, and for his suggestions and encouragement during the investigation.

This technical report has been reviewed and is approved.

A. A. THOMAS, MD
Director
Toxic Hazards Division
Aerospace Medical Research Laboratory

*Captain Damm's present address: AFWL/LRL, Kirtland AFB, NM 87117.

SECTION I

INTRODUCTION

A program was undertaken in recent years at the Aerospace Medical Research Laboratory to determine the environmental pollution potential of beryllium compounds. Two years ago research was initiated in this laboratory to determine the effects of water-soluble beryllium compounds such as beryllium sulfate on water and on aquatic species. In the initial phase of this research, the interaction of beryllium sulfate solutions with hard and soft water was evaluated (ref 1). The toxicity to guppies of aqueous beryllium sulfate solutions, with differences noted between hard and soft water, was demonstrated using standard bioassay techniques; the results are to be reported elsewhere (ref 2). In order to gain some insight into beryllium toxicity in fish, a separate two-part study was undertaken early in the program in which isotopic tracers were added to beryllium solutions and then applied to two bioassays, respectively.

This report describes the first part of the investigation to monitor beryllium toxicity in guppies by the use of isotopic beryllium (^{7}Be). This phase, preliminary to the biological study, is concerned mainly with the determination of tracer activity (or efficiency) under various conditions, counting geometry relative to the guppy, and the preparations and procedures necessary to measure small amounts of beryllium absorbed by the fish from aqueous solutions of beryllium sulfate. The results of the follow-on phase consisting of biological data obtained in two tracer-applied bioassays are described in a subsequent report (ref 3).

SECTION II

EXPERIMENTAL

METHODS AND MATERIALS

Analytical methods and materials (including equipment, hard and soft water, and beryllium sulfate solutions) used in conjunction with this study are described fully in a previous report (ref 1). Information concerning the test organism (the guppy), aquaria materials, and bioassay procedures are presented in the subsequent report that deals with the biological side of this tracer study (ref 3).

It should be restated here that the beryllium sulfate used was in the form of $BeSO_4 \cdot 4H_2O$, which was obtained from Alfa Inorganics, Inc. (Beverly, Mass.). Beryllium solutions were prepared by dissolving this salt in hard water (400 mg/l as $CaCO_3$) or soft water (20 mg/l). Throughout this study the term $BeSO_4$ or beryllium solution refers to the aqueous solution of $BeSO_4.4H_2O$, and beryllium concentrations are expressed in terms of the metal ion (Be^{2+}).

The beryllium isotope (^7Be) was in the form of $BeCl_2$ (in 0.5N HCl, 0.4 mCi/ml and 99.9% radiometric purity) and was obtained from New England Nuclear (Boston, Mass.). The isotope was added to each test jar containing $BeSO_4$ solution to give an amount of radioactivity not exceeding 0.04 microcurie/milliliter. In a pilot study, this low amount of radioactivity in hard and soft water had no effect on guppies for a period up to 1 week.

Preparation of the beryllium standard and water samples with the isotope follows. The original ^7Be solution was diluted by a factor of 10^4, so that one milliliter standard $^7Be = 4 \times 10^{-2}$ microcurie; this standard exhibited 1480 disintegrations per second per milliliter. Each water sample (i.e., beryllium sulfate solution) of 2-liter volume was injected with 2 ml of 1:10 dilution of original isotope (or 0.2 ml of

undiluted isotope). This yielded 0.04 μCi per ml test solution, the same radioactivity level as the isotope standard.

The glassware used for containing the $BeSO_4$ solutions and the fish for bioassay work consisted of 1-gallon jars (obtained from The Cincinnati Container Co., Cincinnati, Ohio). This glassware was made of soda lime silica (composed mainly of 72% SiO_2, 13% Na_2O, and 10% CaO, and less inert chemically than borosilicate glass) and was compared to polyethylene for its suitability as a container of $BeSO_4$ solutions. Loss of the metal from solution was evaluated at the high and low concentrations levels of Be^{2+} used in the bioassays. $BeSO_4$ solutions were placed in the gallon-size jars with and without a polyethylene sheet liner.

RADIOACTIVITY MEASUREMENTS

Radioactivity was measured in a scintillation counter with well detector containing a sodium iodide (NaI) crystal. Excitation of the NaI crystal produces flashes of light that are picked up by a photomultiplier tube, signals from which are amplified, screened, shaped, and then counted in a scaler-timer.

Radioactive counts were taken on the media ($BeSO_4$ solution), the fish (when applicable), and a standard vial of ^7Be. Half-life corrections to all measurements were obtained by reading the standard vial daily. Counting of all radioactivity in a narrow energy band centered at 0.48 Mev gamma energy was done for the same period of time (e.g., 100, 256, or 512 seconds) in each experiment so as to cut down on the high and low energy background. The ratio of counts of sample (Be solution or fish) to counts of standard, after background correction, equals the relative activity of the sample, a major measurement in this study.

SECTION III

RESULTS AND DISCUSSION

Small concentrations of beryllium can be effectively monitored in various media and living organisms by means of a radioactive tracer. Radioactive beryllium, ^{7}Be, is ideal for this purpose because of its decay rate (53-day half-life) and its gamma energy (0.48 Mev). Throughout this program (ref 1-3), beryllium (as $BeSO_4$) has been evaluated chemically or biologically in a concentration range from 10^{-2} to 10^{2} mg/l Be^{2+}. In this study, tracer was added in equal amounts to each beryllium test solution; this permitted a very sensitive measure of the most minute concentration. If a direct radioactive-to-cold ratio were used in contrast to a constant tracer level for all solutions, radioactivity would vary by a factor of 10^{4} (i.e., activity at 100 mg/l Be^{2+} being 10^{4} times that at 0.01 mg/l Be^{2+}); thus count times would necessarily vary by such an amount also. The exact ^{7}Be concentration in each solution was not critical since (1) it was very small: $< 1.1 \times 10^{-7}$ mg ^{7}Be/liter, which did not affect the total Be^{2+} level; and (2) ^{7}Be activity was monitored in a relative, not absolute, measure.

Preliminary studies with the tracer were undertaken first to insure that isotope activity is proportional to isotope concentration, second to ascertain if, and to what extent, beryllium is lost from solution over a certain time, third to determine the counting geometry and other variables that may affect counting efficiency without and with the test organism, and fourth to establish procedures for estimating beryllium concentrations in exposed fish and/or their environment.

In the first experiment, radioactive counts were taken for 20-minute periods at six different concentrations of isotope (tracer diluted with water from 1:10,000 to 1:500,000). The results showed a linear relationship between isotope activity and isotope concentration. In a similar experiment, fresh isotope solution was compared with that stored in glass

for 8 days. Radioactivity, which was measured per 512-second period at the same six isotope levels as previously, was directly related to tracer concentration in both cases, as shown in figure 1. It is also evident from the radioactivity data plotted in this figure that the amount of beryllium isotope in solution stored in glass for 8 days was almost identical to that of the fresh solution.

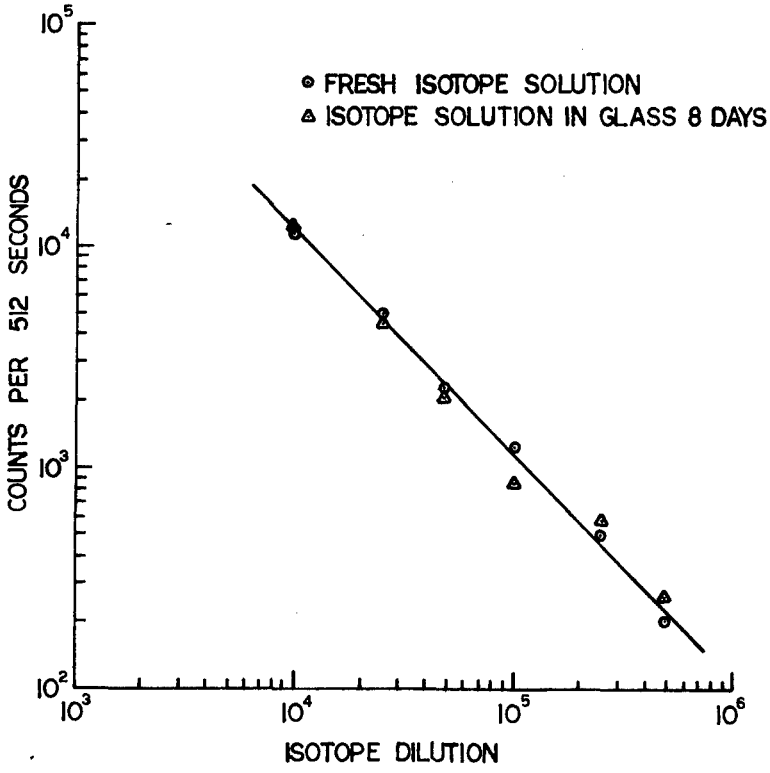

Figure 1. Detected Activity (Per 512-Second Period) Versus Isotope Dilution of Fresh and Stored Tracer Solutions.

It has been reported in the literature that beryllium at certain concentrations is adsorbed onto the walls of glass containers. Untreated borosilicate glass, for example, adsorbed from 8 to 12% of the beryllium present in solution at the 10^{-8} g/ml concentration (ref 4); this amount is equivalent to the lowest level tested in our program (0.01 mg/l Be^{2+}). Losses due to adsorption of ionic or molecular species of beryllium become more significant at this low concentration than at higher levels (ref 4, 5).

In addition to the importance of the type of container used for beryllium work (e.g., borosilicate versus Teflon vials) and the degree of dilution of this metal in solution, more recent work has indicated that the nature of the beryllium compound and the storage (or contact) time in a reaction vessel are also factors to be considered in the accuracy of beryllium analysis.[1] For example, for the same period of time, losses (due to glass adsorption) observed for very low concentrations of beryllium trifluoro-acetylacetonate (ref 4) were absent for equally diluted $Be(NO_3)_2$ solutions, and the former did not show appreciable losses until after 7 days.[1] Thus, experiments were conducted to determine if beryllium would be lost from $BeSO_4$ solutions under the conditions of our study and/or if the glass containers obtained for bioassay work have to be treated before use, such as acid washed (ref 6), silanized, or Teflon-coated (ref 4).

One-gallon bioassay jars were divided into two groups: half were lined with a polyethylene sheet and half were not. Two-liter solutions of $BeSO_4$ were made up at two different concentrations, 10^{-2} and 10^{2} mg/l Be^{2+}, and distributed evenly between the two types of containers. Isotopic beryllium (100 microliters ^{7}Be/liter) was added to each jar. Two to three ml samples in duplicate from each jar were analyzed (counted) three times a day for 4 days (a bioassay period) and once for 8 days. The mean daily count ratios with standard deviation for high and low levels of Be^{2+} in glass versus polyethylene are presented in table I. The results showed no significant difference between polyethylene and glass containers of $BeSO_4$ solution in the 10^{-2} to 10^{2} mg/l Be^{2+} range for a period of 4 days. Moreover, additional data showed similarly no loss of isotope from solution in glass jars stored for a period up to 8 days (see, e.g., fig. 1). When the tabulated count ratios are plotted versus time for each of the four test conditions, the results are shown in figure 2. No significant difference exists between polyethylene and glass from one day to the next within a

---

[1]Eisentraut, K.J., Aerospace Research Laboratory, Wright-Patterson Air Force Base, Ohio: Personal communication.

relatively short period of time. Contrary to observing losses of Be from solution onto container walls, there appeared to be a slight increase of Be^{2+} in solution with time; however, this apparent difference was not real due to the overlap of count ratios within one standard deviation between Day 0 and Day 4. Thus, these results indicate that beryllium was not lost from the environment under the conditions of this study by adsorption onto the container walls or by evaporation (cf., figs. 1, 2).

TABLE I

EVALUATION OF BERYLLIUM SULFATE - LABELLED SOLUTIONS IN GLASS VERSUS POLYETHYLENE CONTAINERS

Be^{2+} Concn (mg/l)	Container	^{7}Be Radioactive Counts*				
		Day 0	Day 1	Day 2	Day 3	Day 4
100	Polyethylene	1.18±0.04	1.20±0.03	1.19±0.03	1.24±0.03	1.25±0.03
	Glass	1.18±0.07	1.18±0.04	1.21±0.04	1.24±0.04	1.22±0.03
0.01	Polyethylene	1.17±0.04	1.15±0.05	1.14±0.04	1.19±0.03	1.20±0.04
	Glass	1.15±0.03	1.16±0.06	1.17±0.03	1.16±0.03	1.19±0.05

*Sample/standard ratios

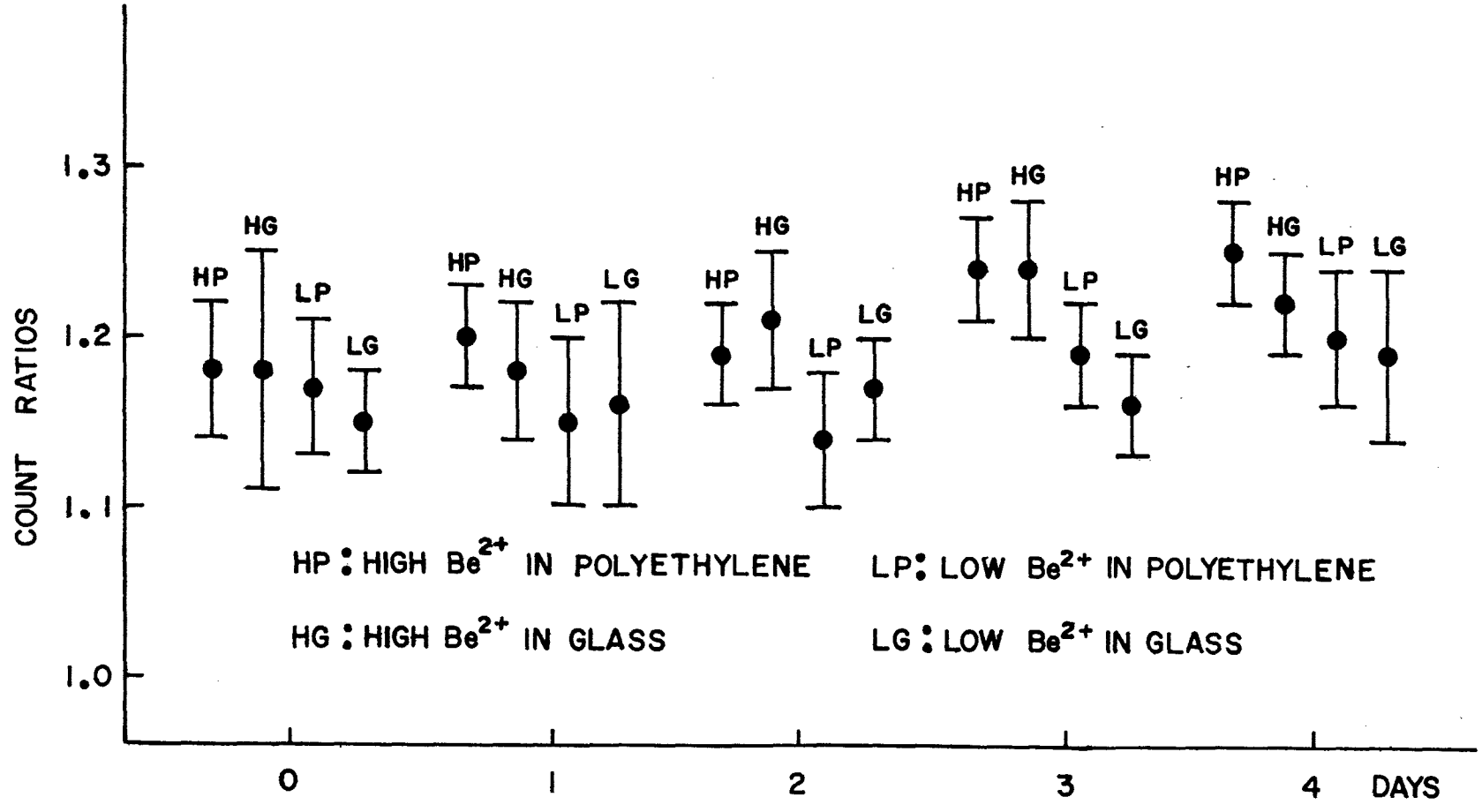

Figure 2. Comparison of the Retention of $BeSO_4$ Solutions (Injected with Isotope) Between Polyethylene and Glass Containers Over Time. Mean count ratios of several daily duplicate samples are plotted versus time in days with the standard deviation represented by vertical lines. Count time: 256 second periods.

In the next experiments, variables in counting geometry for the 3- by 3-inch NaI well detector were examined. One-milliliter aqueous samples each containing ^{7}Be isotope diluted 1:10,000 with distilled water were further diluted (with 0-9 ml distilled water) to various volumes from 1 to 10 milliliters. The number of counts (per 512-second period) was plotted against each of the six dilution volumes used in the well. The results (see fig. 3) showed no difference in isotope activity (within ± 4% of the mean) with the different sample volumes; thus, counting efficiency was independent of dilution volume of equal activity aqueous samples. In another experiment, 1-milliliter aqueous samples, each containing 10^{4}-diluted isotope, were examined at various positions in the well detector. The 1-ml samples were counted (per 512-second period) at five levels ranging from 0 to 1.5 inches above well bottom. The results, plotted in figure 4, showed only a small difference in counting activity (within ± 20% of the mean) between a sample at well bottom and one positioned 1-1/2 inches above bottom, indicating that counting efficiency was relatively independent of the position of equal activity water samples in the well detector. These two sets of experiments showed that the "relative" activity (amount) of the beryllium ingested or adsorbed by an organism can be determined directly without geometry corrections no matter if the test fish are (a) of unequal size (volume or weight) and (b) not positioned consistently in bottom of detector well. Furthermore, this indicates that water samples and exposed fish can be treated alike as far as the detector geometry is concerned. We assumed that the organisms do not selectively ingest one particular beryllium isotope over another and that (barring ^{7}Be decay) tracer-to-cold Be ratios, ^{7}Be:^{9}Be, remain constant in tissue and water alike.

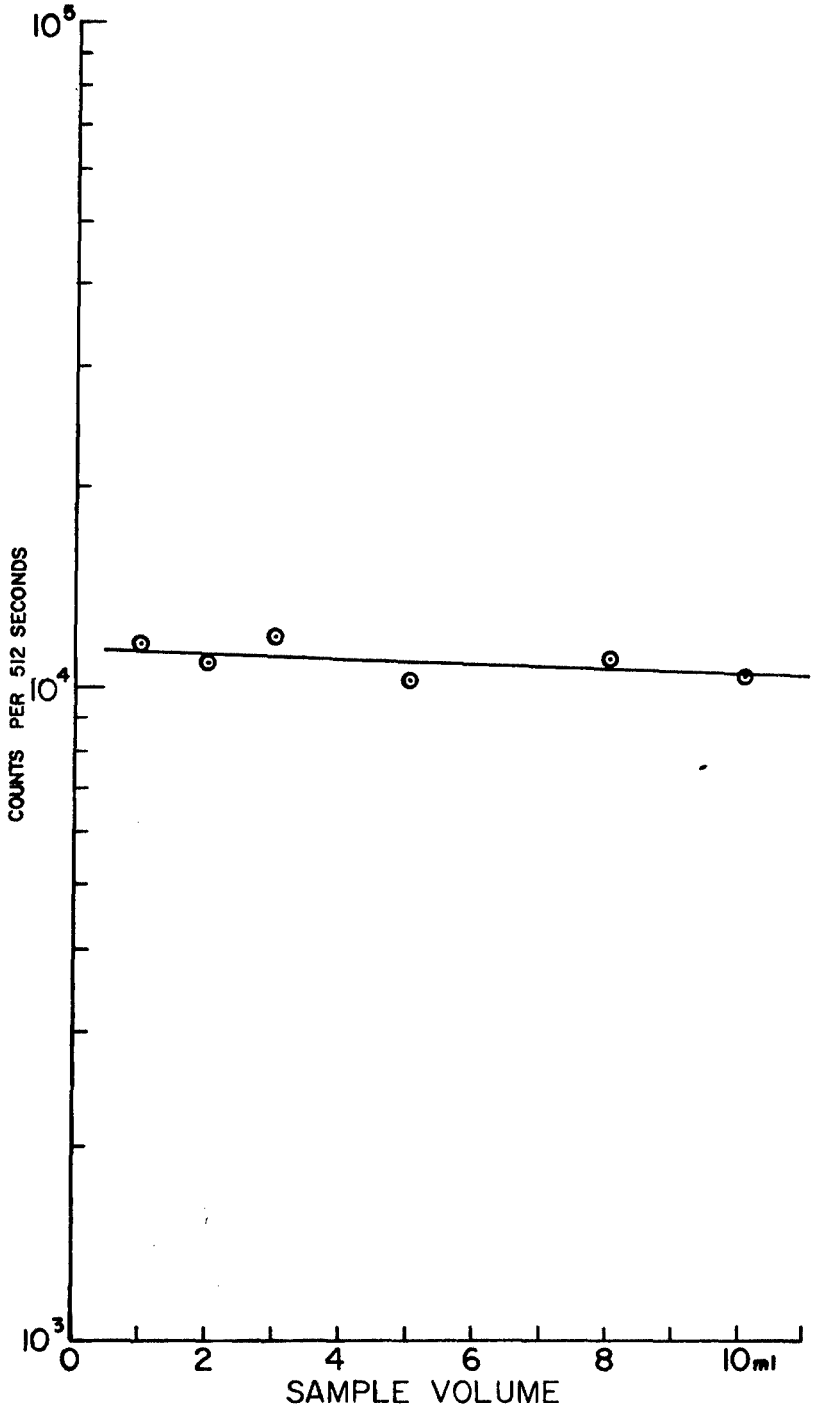

Figure 3. Detected Activity of Beryllium Isotope Versus Sample Volume. One ml solution of isotope ($1:10^4$ dilution) further diluted with water to different volumes and counted in well detector.

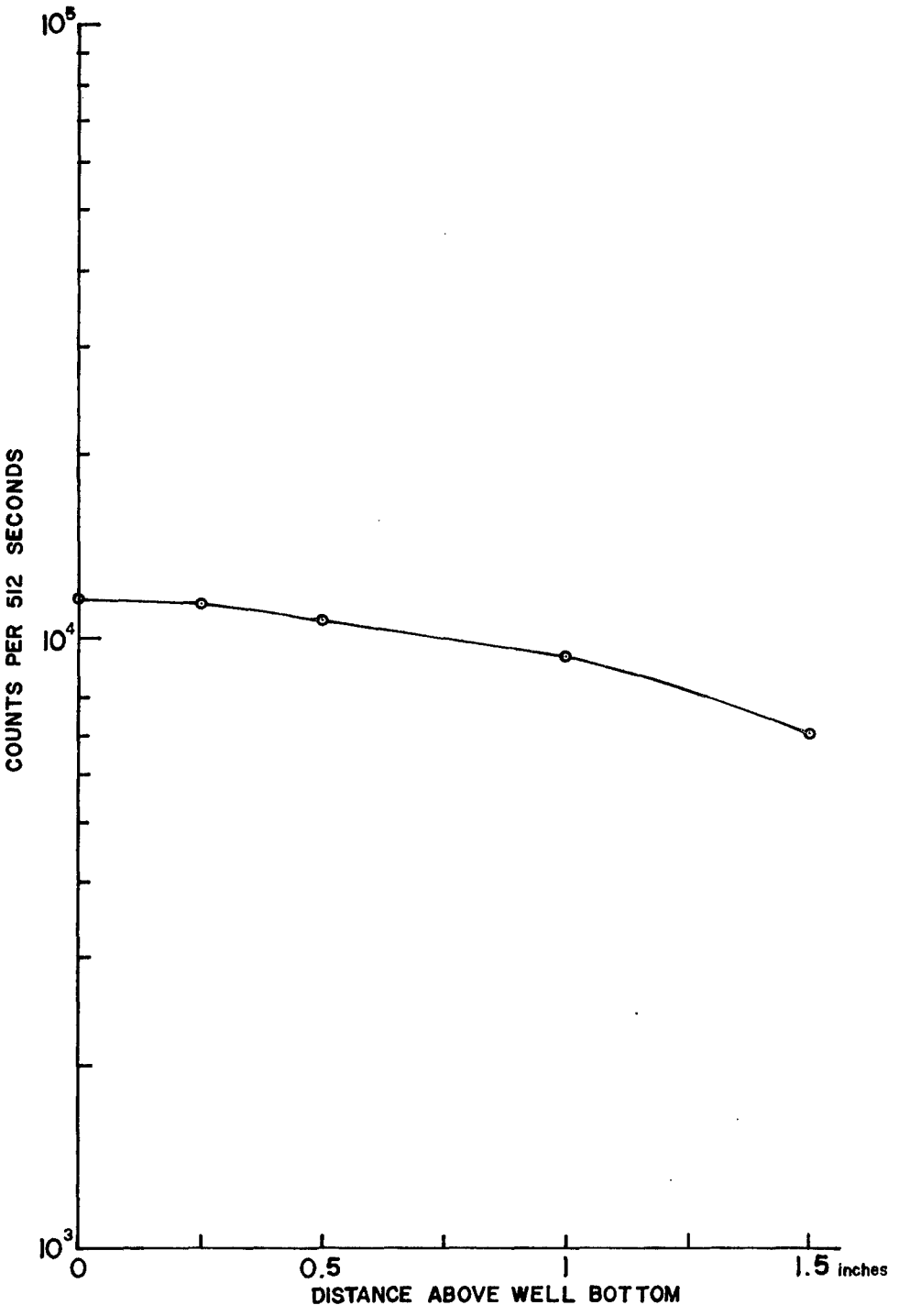

Figure 4. Detected Activity of Beryllium Isotope Versus Position in Well Detector. One ml solution of 10^4-diluted ^7Be at various positions in well detector.

The half-life of ^{7}Be is 53 days. The use of decay correction factors in an extended experimental period can be avoided by preparing a ^{7}Be standard with which activities of unknown samples are compared. Through each experiment, the activity of the standard falls at the same rate as the unknown. The ratio of activity of unknown, U, to that of the standard, S, will always remain constant, as shown by the following equations:

$$S = S_o e^{-\lambda t} \tag{1}$$

where S_o = initial activity of standard

λ = decay constant for ^{7}Be

t = time from point of initial activity

$$U = U_o e^{-\lambda t} \tag{2}$$

where U_o = initial activity of unknown

$$\text{thus } \frac{U}{S} = \frac{U_o e^{-\lambda t}}{S_o e^{-\lambda t}} = \frac{U_o}{S_o} = \text{constant} \tag{3}$$

The amount of beryllium taken up by the fish from their environment is determined by making radioactivity measurements on fish, water, and standard at the same time, correcting for background activity, and then standardizing the counts. From the standardized counts and weights of the fish or tissues in question, the beryllium concentration values are calculated. Thus, the concentration of beryllium in fish or organ is estimated according to the following procedure (table II):

TABLE II

PROCEDURE FOR ESTIMATING BERYLLIUM IN FISH

1. Terms:
   (a) N_w = total (scaler) counts in 1 ml water samples (per 100 sec)
   (b) N_f = total counts in dead fish (100 sec)
   (c) N_s = total counts in standard (100 sec); this value (N_s) is taken at the same time that N_w or N_f counts are taken to allow for decay correction
   (d) B = background counts (100 sec); this value is taken at the same time as N_w, N_f, or N_s
   (e) C = concentration of $BeSO_4$ in solution in terms of Be^{2+}, mg/l
   (f) X = concentration of Be^{2+} per unknown sample in micrograms (µg)
   (g) W = weight of fish or organ in grams (g)

2. To correct for background radioactivity:
   (a) Background-corrected water counts, $N_w' = N_w - B$
   (b) Background-corrected fish counts, $N_f' = N_f - B$
   (c) Background-corrected standard counts, $N_s' = N_s - B$

3. To standardize counts on fish and environment:
   (a) Standardized water count (per ml), $K_w = \frac{N_w'}{N_s'}$; this value is independent of background and decay activity
   (b) Standardized fish count, $K_f = \frac{N_f'}{N_s'}$

4. To calculate beryllium uptake:
   (a) Be uptake per sample, $X = \frac{K_f C}{K_w}$, expressed as microgram Be^{2+} per unknown sample
   (b) Be uptake per gram of sample, $X' = \frac{X}{W}$, expressed as microgram Be^{2+} per gram of fish or organ

REFERENCES

1. Slonim, A. R., Behavior of Beryllium Sulfate in Hard and Soft Water, AMRL-TR-71-100, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio, December 1971.

2. Slonim, A. R., "Acute Toxicity of Beryllium Sulfate to the Common Guppy," 1972, Submitted to journal (AMRL-TR-72-122).

3. Slonim, A. R., and F. C. Damm, Beryllium Uptake by the Common Guppy Using Radioisotope ^{7}Be. II. Beryllium Concentration in Fish, AMRL-TR-72-95, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio, October 1972.

4. Ross, W. D., and R. E. Sievers, "Ultra-Trace Analysis of Beryllium by Gas Chromatography," Proc. Sixth Intern. Symp. on Gas Chromatography and Associated Techniques, Rome, 20-23 September 1966. In: Gas Chromatography 1966 (A. B. Littlewood, Ed.), Inst. of Petroleum (Elsevier), London, 1967, p 272.

5. Ross, W. D., and R. E. Sievers, "Rapid Ultra-Trace Determination of Beryllium by Gas Chromatography," Talanta, 15, 87, 1968.

6. Keenan, R. G., "Analytical Determination of Beryllium," Chap. 5, In: Beryllium - Its Industrial Hygiene Aspects, (H. E. Stokinger, Ed.), Academic Press, New York, 1966, p 133.

☆U.S.Government Printing Office: 1973 — 759-491/405